Medline

Separation of scrapie prion infectivity from PrP amyloid polymers.

Wille H, Zhang GF, Baldwin MA, Cohen FE, Prusiner SB

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Abstract

The prion protein (PrP) undergoes a profound conformational change when the cellular isoform (PrPC) is converted into the scrapie form (PrPSc). Limited proteolysis of PrPsc produces PrP 27-30 which readily polymerizes into amyloid. To study the structure of PrP amyloid, we employed organic solvents that perturb protein conformation. Hexafluoro-2-propanol (HFIP), which promotes alpha-helix formation, modified the ultrastructure of rod-shaped PrP amyloids; flattened ribbons with a more regular substructure were found. As the concentration of HFIP was increased, the beta-sheet content and proteinase K resistance of PrP 27-30 as well as prion infectivity diminished. HFIP reversibly decreased the binding of Congo red dye to the rods while inactivation of prion infectivity was irreversible. In contrast to 10% HFIP, 1,1,1-trifluoro-2-propanol (TFIP) did not inactivate prion infectivity but like HFIP, TFIP did alter the morphology of the rods and abolish Congo red binding. This study separates prion infectivity from the amyloid properties of PrP 27-30 and underscores the dependence of prion infectivity on PrPSc conformation. The results also demonstrate that the specific beta-sheet-rich structures required for prion infectivity can be differentiated from those needed for amyloid formation as determined by Congo red binding.

MeSH

1-Propanol; Acetone; Alcohols; Animal; Congo Red; Electrophoresis, Polyacrylamide Gel; Endopeptidase K; Female; Fluorocarbons; Glycerol; Hamsters; Microscopy, Electron; PrP 27-30 Protein; PrPC Proteins; Protein Conformation; Protein Structure, Secondary; Scrapie; Serine Endopeptidases; Solubility; Solvents; Spectrophotometry; Spectroscopy, Fourier Transform Infrared; Sucrose; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Author Address

Department of Neurology, University of California, San Francisco 94143,

of this method is greatly enhanced when combined with PCR [Wrichnik et al, Nucleic Acids Res. 15:529-542 (1987); Wong et al, Nature 330:384-386 (1987); Stoflet et al, Science 239:491-494 (1988)]. In the latter procedure, a sequencing primer which lies within the amplified sequence is used with double-stranded PCR product or single-stranded template generated by a modified PCR. The sequence determination is performed by conventional procedures with radiolabeled nucleotides or by automatic sequencing procedures with fluorescent-tags.

Sequence alterations may occasionally generate fortuitous restriction enzyme recognition sites which are revealed by the use of appropriate enzyme digestion followed by conventional gel-blot hybridization (Southern, J. Mol. Biol 98: 503 (1975)). DNA fragments carrying the site (either normal or mutant) are detected by their reduction in size or increase of corresponding restriction fragment numbers. Genomic DNA samples may also be amplified by PCR prior to treatment with the appropriate restriction enzyme; fragments of different sizes are then visualized under UV light in the presence of ethidium bromide after gel electrophoresis.

Genetic testing based on DNA sequence differences may be achieved by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing reagent. Small sequence deletions and insertions can be visualized by high resolution gel electrophoresis. For example, the PCR product with the 3 bp deletion is clearly distinguishable from the normal sequence on an 8% non-denaturing polyacrylamide gel. DNA fragments of different sequence compositions may be distinguished on denaturing formamide gradient gel in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific "partial-melting" temperatures (Myers, supra). In addition, sequence alterations, in particular small deletions, may be detected as changes in the